

10/5/6439

AP20Rec'd PCT/PTO 20 APR 2006

SELECTION AND TREATMENT OF PATIENTS SUFFERING FROM WASTING

FIELD OF THE INVENTION

The invention relates to growth hormone (GH)
5 secretagogues, such as GH releasing factor (GRF) and analogs
thereof, and uses thereof.

BACKGROUND OF THE INVENTION

Wasting is a severe clinical condition associated
with various diseases. Generally, this condition is
10 characterized by a certain degree of involuntary weight loss,
associated with a loss in lean body mass, whereas fat mass
may decrease, increase or remain stable. In these patients,
muscle wasting is very often associated with a poor prognosis
and limited survival expectation, independently of the
15 severity of the underlying disease.

An example of a wasting-type of condition is
chronic obstructive pulmonary disease (COPD). COPD is
characterized by progressive airflow obstruction due to
chronic bronchitis or emphysema. Weight loss and muscle
20 wasting are independent predictors of functional capacity and
mortality in these patients (Schols et al. 1998).
Consequently, anabolic interventions have been considered in
order to promote muscle gain and muscle function. Nutritional
repletion (Ferreira et al., 2000), anabolic steroids
25 (Weisberg et al., 2002; Ferreira et al., 1998) as well as
short term (3 weeks) administration of growth hormone (Burdet
et al., 1997) have shown limited success particularly on
muscle function. Although much attention is given to the
pulmonary problems (emphysema, chronic bronchitis), it has
30 become more and more a clinical reality in the past recent

years that COPD is a multi-component disease, implying two major components, the pulmonary and the periphery (muscle mass). The evolution of these two components is not parallel. Several prospective and retrospective studies have clearly demonstrated that involuntary weight loss or a loss in lean body mass are independent predictors of mortality, and are associated with diminished endurance exercise capacity, impaired quality of life and increase utilization of health care costs.

Because of its anabolic effects and the decrease of its secretion in aging, growth hormone (GH) has been the subject of numerous clinical trials aiming at increasing muscle mass and functionality in several clinical conditions. Most of the clinical studies aiming at improving muscle mass and function in patients by a growth hormone replacement therapy have been conducted with recombinant human GH (rhGH), and resulted most frequently in increases in lean body mass, both in healthy volunteers and special patient populations. However, these changes in body composition have resulted in very inconsistent changes in muscle function, which is a desired clinical outcome. In that regard, Zachwieja et al. (1999) reviewed a series of studies done in healthy older volunteers, showing consistent increases in lean body mass, that were only rarely associated with increases in muscle strength. In COPD patients, two studies have been conducted with recombinant growth hormone. Both of them resulted in significant increases in lean body mass, but one of them (Pape et al., 1991) reported functional improvement, only in the form of an improved maximal inspiration pressure (PI_{max}), whereas the other (Burdet et al, 1997) failed to show any change in PI_{max} , handgrip strength or exercise capacity.

Another condition associated with wasting is HIV infection or AIDS. In these particular conditions, administration of recombinant GH has shown positive effects, both on body composition and on exercise performance
5 (Schambelan et al., 1996).

Therefore, it appears from the scientific literature that GH replacement therapy in patients with various degrees of wasting most often results in important changes in body composition towards an increase in muscle
10 mass, but in variable results in terms of muscle functionality and clinical status. The variability in functional outcome of anabolic therapy is critical to the success of the therapy to the patient suffering from cachexia or wasting. There is thus a great need for therapeutics capable of restoring in
15 wasting patients, not only muscle mass, but also muscle function.

SUMMARY OF THE INVENTION

The invention relates to GH secretagogues (e.g. GRF
20 and analogs thereof) and uses thereof.

In a first aspect, the present invention provides a method of increasing muscle function in a subject, said method comprising administering to said subject an agent selected from the group consisting of (a) a growth hormone
25 (GH) secretagogue and (b) a composition comprising a GH secretagogue and a pharmaceutically acceptable carrier. In an embodiment, the GH secretagogue is selected from the group consisting of GH-releasing factor (GRF) and a GRF analog. In another embodiment, the GRF analog is a GRF analog of formula
30 A:

X-GRF Peptide (A)

wherein;

5 the GRF peptide is a peptide of formula B;

A1-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-Tyr-Arg-Lys-A13-Leu-
A15-Gln-Leu-A18-Ala-Arg-Lys-Leu-Leu-A24-A25-Ile-A27-A28-
Arg-A30-R0 (B)

10

wherein,

A1 is Tyr or His;

A2 is Val or Ala;

15 A8 is Asn or Ser;

A13 is Val or Ile;

A15 is Ala or Gly;

A18 is Ser or Tyr;

A24 is Gln or His;

20 A25 is Asp or Glu;

A27 is Met, Ile or Nle

A28 is Ser or Asn;

A30 is a bond or amino acid sequence of 1 up to 15
residues; and

25

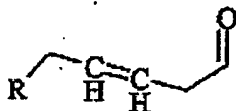
R0 is NH_2 or $\text{NH}-(\text{CH}_2)_n-\text{CONH}_2$, with $n=1$ to 12; and

X is a hydrophobic tail anchored via an amide bond to
the N-terminus of the peptide and the hydrophobic tail
30 defining a backbone of 5 to 7 atoms;

wherein the backbone can be substituted by C_{1-6} alkyl, C_{3-6}
cycloalkyl, or C_{6-12} aryl and the backbone comprises at
least one rigidifying moiety connected to at least two
35 atoms of the backbone;

said moiety selected from the group consisting of double bond, triple bond, saturated or unsaturated C₃₋₉ cycloalkyl, and C₆₋₁₂ aryl.

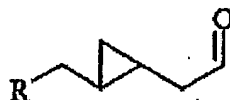
- 5 In a further embodiment, X is selected from the group consisting of:



1 (R=H or CH₃ or CH₂CH₃)
cis or trans



2 (R=H or CH₃ or CH₂CH₃)



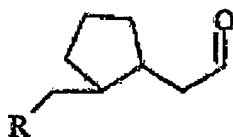
3 (R=H or CH₃ or CH₂CH₃)
cis or trans, both as racemic mixtures



4 (R=H or CH₃ or CH₂CH₃)
cis or trans, both as racemic mixtures
or pure enantiomeric pairs



5 (R=H or CH₃ or CH₂CH₃)
cis or trans, (when R ≠ H)



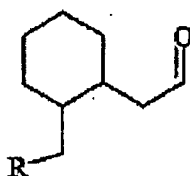
6 (R=H or CH₃ or CH₂CH₃)
cis or trans, both as racemic mixtures
or pure enantiomeric pairs



7 (R=H or CH₃ or CH₂CH₃)

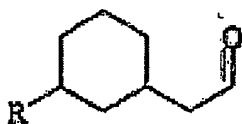
cis or *trans*, (when R \neq H)

both as racemic mixtures
or pure enantiomeric pairs



8 (R=H or CH₃ or CH₂CH₃)

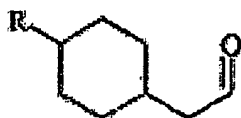
cis or *trans*, both as racemic mixtures
or pure enantiomeric pairs



9 (R=H or CH₃ or CH₂CH₃)

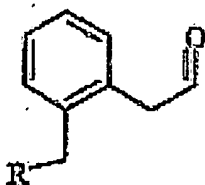
cis or *trans*, (when R \neq H)

both as racemic mixtures
or pure enantiomeric pairs

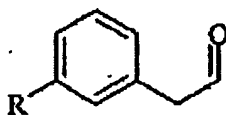
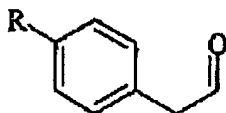


10 (R=H or CH₃ or CH₂CH₃)

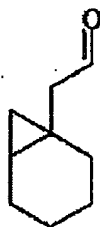
cis or *trans*, (when R \neq H)



11 (R=H or CH₃ or CH₂CH₃)

12 (R=H or CH₃ or CH₂CH₃)13 (R=H or CH₃ or CH₂CH₃)

and



14

In yet another embodiment, A30 is selected from the group consisting of (a) a bond, (b) an amino acid sequence corresponding to positions 30-44 of a natural GRF peptide, and (c) the amino acid sequence of (b) having a 1-14 amino acid deletion from its C-terminus. In yet a further embodiment, the GRF peptide is selected from the group consisting of (a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, (b) a polypeptide comprising the amino acid sequence of SEQ ID NO: 5; and (c) the polypeptide of (a) having a 1 to 14 amino acid deletion from its C-terminus. In embodiments, the GRF analog is (hexenoyl trans-3)hGRF(1-44)NH₂ (SEQ ID NO: 7).

In another aspect, the present invention also provides a method for increasing a muscle function wherein the muscle function is selected from the group consisting of (a) muscle strength, (b) muscle endurance and (c) both (a) and (b). In an embodiment, the muscle function is muscle

strength, and in a further embodiment, the muscle strength is peripheral muscle strength. In another embodiment, the muscle function is muscle endurance. In a further embodiment, the increase in muscle function results in a reduction of a parameter selected from the group consisting of (a) breathing discomfort, (b) leg discomfort and (c) both (a) and (b). In yet a further embodiment, the increase results in an increase in lean body mass in the subject and/or a decrease in fat mass in the subject. In another embodiment, the subject suffers from wasting, and in a further embodiment, wasting is associated with a condition selected from the group consisting of chronic obstructive pulmonary disease, chronic renal failure, congestive heart failure, human immunodeficiency virus infection, acquired immunodeficiency syndrome, cancer, malnutrition, frailty, immobilization paraplegia and spinal disorder. In yet another embodiment, the subject suffers from severe wasting. In an embodiment, the subject has a body mass index less than or equal to 20 and/or a weight less than 90% of ideal body weight. In an embodiment, the subject is a male and has a fat free mass index less than or equal to 16 or the subject is a female and has a fat free mass index less than or equal to 15. In embodiments, the agent is administered in a route selected from the group consisting of intravenous, oral, transdermal, subcutaneous, mucosal, intramuscular, intranasal, intrapulmonary, parenteral, intrarectal and topical. In another embodiment, the GH secretagogue is administered in a dose from about 0.0001 mg to about 4 mg, in a further embodiment, from about 0.0001 to about 2 mg, in a further embodiment, from about 1mg to about 2 mg, in a further embodiment, about 1 mg, in a further embodiment, about 2 mg.

In another aspect, the present invention provides use of an agent selected from the group consisting of (a) a

growth hormone (GH) secretagogue and (b) a composition comprising a GH secretagogue and a pharmaceutically acceptable carrier; for increasing muscle function in a subject. In an embodiment, the GH secretagogue is selected from the group consisting of GH-releasing factor (GRF) and a GRF analog. In another embodiment, the GRF analog is a GRF analog of formula A:

X-GRF Peptide (A)

10

wherein;

the GRF peptide is a peptide of formula B;

15

A1-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-Tyr-Arg-Lys-A13-Leu-A15-Gln-Leu-A18-Ala-Arg-Lys-Leu-Leu-A24-A25-Ile-A27-A28-Arg-A30-R0 (B)

wherein,

20

A1 is Tyr or His;

A2 is Val or Ala;

A8 is Asn or Ser;

A13 is Val or Ile;

25

A15 is Ala or Gly;

A18 is Ser or Tyr;

A24 is Gln or His;

A25 is Asp or Glu;

A27 is Met, Ile or Nle

30

A28 is Ser or Asn;

A30 is a bond or amino acid sequence of 1 up to 15 residues; and

R0 is NH₂ or NH-(CH₂)_n-CONH₂, with n=1 to 12; and

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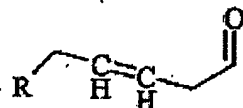
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X is a hydrophobic tail anchored via an amide bond to the N-terminus of the peptide and the hydrophobic tail defining a backbone of 5 to 7 atoms;

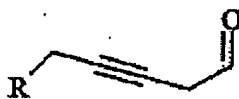
5 wherein the backbone can be substituted by C₁₋₆ alkyl, C₃₋₆ cycloalkyl, or C₆₋₁₂ aryl and the backbone comprises at least one rigidifying moiety connected to at least two atoms of the backbone;

10 said moiety selected from the group consisting of double bond, triple bond, saturated or unsaturated C₃₋₉ cycloalkyl, and C₆₋₁₂ aryl.

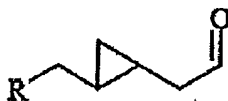
In yet another embodiment, X is selected from the group
15 consisting of:



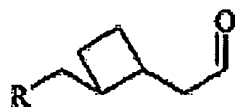
1 (R=H or CH₃ or CH₂CH₃)
cis or trans



2 (R=H or CH₃ or CH₂CH₃)



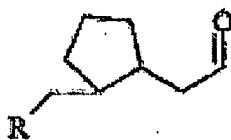
3 (R=H or CH₃ or CH₂CH₃)
*cis or trans, both as racemic mixtures
or pure enantiomeric pairs*



- 4** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, both as racemic mixtures
 or pure enantiomeric pairs



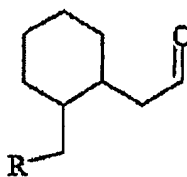
- 5** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, (when $R \neq H$)



- 6** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, both as racemic mixtures
 or pure enantiomeric pairs

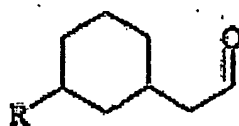
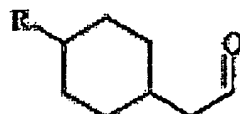
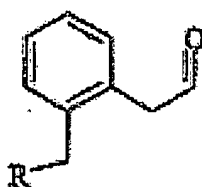
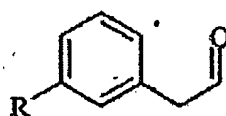
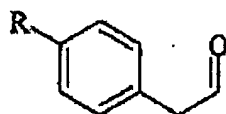


- 7** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, (when $R \neq H$)
 both as racemic mixtures
 or pure enantiomeric pairs



- 8** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, both as racemic mixtures
 or pure enantiomeric pairs

12.

9 (R=H or CH₃ or CH₂CH₃)*cis* or *trans*, (when R ≠ H)both as racemic mixtures
or pure enantiomeric pairs10 (R=H or CH₃ or CH₂CH₃)*cis* or *trans*, (when R ≠ H)11 (R=H or CH₃ or CH₂CH₃)12 (R=H or CH₃ or CH₂CH₃)13 (R=H or CH₃ or CH₂CH₃)

and



14

In yet a further embodiment, A30 is selected from the group consisting of (a) a bond, (b) an amino acid sequence corresponding to positions 30-44 of a natural GRF peptide, and (c) the amino acid sequence of (b) having a 1-14 amino acid deletion from its C-terminus. In yet another embodiment, the GRF peptide is selected from the group consisting of (a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, (b) a polypeptide comprising the amino acid sequence of SEQ ID NO: 5 and (c) the polypeptide of (a) having a 1 to 14 amino acid deletion from its C-terminus. In embodiments, the GRF analog is (hexenoyl trans-3)hGRF(1-44)NH₂ (SEQ ID NO: 7).

In a further aspect, the invention also provides use of an agent for increasing a muscle function, wherein the muscle function is selected from the group consisting of (a) muscle strength, (b) muscle endurance; and (c) both (a) and (b). In an embodiment, the muscle function is muscle strength, and in a further embodiment, peripheral muscle strength. In another embodiment, the muscle function is muscle endurance. In yet another embodiment, the increase in muscle function results in a reduction of a parameter selected from the group consisting of (a) breathing discomfort, (b) leg discomfort and (c) both (a) and (b). In a further embodiment, the increase in muscle function results in an increase in lean body mass in the subject and/or a decrease in fat mass in the subject. In another embodiment, the subject suffers from wasting, and in a further embodiment, wasting is associated with a condition selected from the group consisting of chronic obstructive pulmonary disease, chronic renal failure, congestive heart failure, human immunodeficiency virus infection, acquired immunodeficiency syndrome, cancer, malnutrition, frailty, immobilization paraplegia and spinal disorder. In yet

another embodiment, the subject suffers from severe wasting. In an embodiment, the subject may have a body mass index less than or equal to 20 and/or a weight less than 90% of ideal body weight. In an embodiment, the subject is a male and has a fat free mass index less than or equal to 16 or the subject is a female and has a fat free mass index less than or equal to 15. In another embodiment, the agent is adapted for an administration route selected from the group consisting of intravenous, oral, transdermal, subcutaneous, mucosal, intramuscular, intranasal, intrapulmonary, parenteral, intrarectal and topical. In a further embodiment, the GH secretagogue is adapted for administration in a dose from about 0.0001 mg to about 4 mg, in a further embodiment, from about 0.0001 to about 2 mg, in a further embodiment, from about 1mg to about 2 mg, in a further embodiment, about 1 mg, in a further embodiment, about 2 mg.

In yet another aspect, the present invention provides use of an agent selected from the group consisting of (a) a growth hormone (GH) secretagogue and (b) a composition comprising a GH secretagogue and a pharmaceutically acceptable carrier; for the manufacture of a medicament for increasing muscle function in a subject.

In a further aspect, the present invention provides a package comprising (i) an agent selected from the group consisting of (a) a growth hormone (GH) secretagogue and (b) a composition comprising a GH secretagogue and a pharmaceutically acceptable carrier; and (ii) instructions for increasing muscle function in a subject. In an embodiment, the GH secretagogue is selected from the group consisting of GH-releasing factor (GRF) and a GRF analog. In another embodiment, the GRF analog is a GRF analog of formula A:

15

X-GRF Peptide (A)

wherein;

5 the GRF peptide is a peptide of formula B;

A1-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-Tyr-Arg-Lys-A13-Leu-
A15-Gln-Leu-A18-Ala-Arg-Lys-Leu-Leu-A24-A25-Ile-A27-A28-
Arg-A30-R0 (B)

10

wherein,

A1 is Tyr or His;

A2 is Val or Ala;

15 A8 is Asn or Ser;

A13 is Val or Ile;

A15 is Ala or Gly;

A18 is Ser or Tyr;

A24 is Gln or His;

20 A25 is Asp or Glu;

A27 is Met, Ile or Nle

A28 is Ser or Asn;

A30 is a bond or amino acid sequence of 1 up to 15
residues; and

25

R0 is NH₂ or NH-(CH₂)_n-CONH₂, with n=1 to 12; and

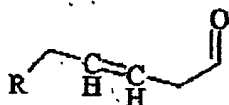
X is a hydrophobic tail anchored via an amide bond to
the N-terminus of the peptide and the hydrophobic tail
30 defining a backbone of 5 to 7 atoms;

wherein the backbone can be substituted by C₁₋₆ alkyl, C₃₋₆
cycloalkyl, or C₆₋₁₂ aryl and the backbone comprises at
least one rigidifying moiety connected to at least two
35 atoms of the backbone;

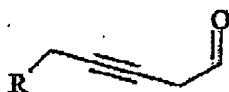
said moiety selected from the group consisting of double bond, triple bond, saturated or unsaturated C₃₋₉ cycloalkyl, and C₆₋₁₂ aryl.

5

In yet another embodiment, X is selected from the group consisting of:



1 (R=H or CH₃ or CH₂CH₃)
cis or *trans*



2 (R=H or CH₃ or CH₂CH₃)



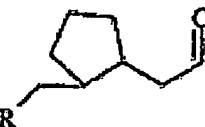
3 (R=H or CH₃ or CH₂CH₃)
cis or *trans*, both as racemic mixtures
or pure enantiomeric pairs



4 (R=H or CH₃ or CH₂CH₃)
cis or *trans*, both as racemic mixtures
or pure enantiomeric pairs



5 (R=H or CH₃ or CH₂CH₃)
cis or *trans*, (when R ≠ H)



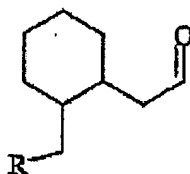
6 (R=H or CH₃ or CH₂CH₃)
cis or *trans*, both as racemic mixtures
or pure enantiomeric pairs



7 (R=H or CH₃ or CH₂CH₃)

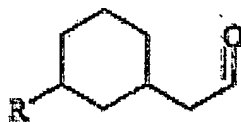
cis or *trans*, (when R \neq H)

both as racemic mixtures
or pure enantiomeric pairs



8 (R=H or CH₃ or CH₂CH₃)

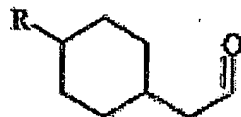
cis or *trans*, both as racemic mixtures
or pure enantiomeric pairs



9 (R=H or CH₃ or CH₂CH₃)

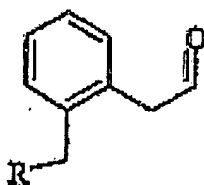
cis or *trans*, (when R \neq H)

both as racemic mixtures
or pure enantiomeric pairs

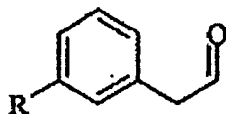
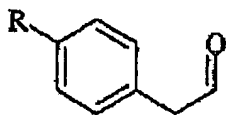
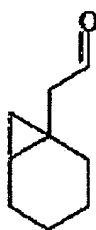


10 (R=H or CH₃ or CH₂CH₃)

cis or *trans*, (when R \neq H)



11 (R=H or CH₃ or CH₂CH₃)

12 (R=H or CH₃ or CH₂CH₃)13 (R=H or CH₃ or CH₂CH₃) and

14

In yet a further embodiment, A30 is selected from the group consisting of (a) a bond, (b) an amino acid sequence
 5 corresponding to positions 30-44 of a natural GRF peptide, and (c) the amino acid sequence of (b) having a 1-14 amino acid deletion from its C-terminus. In yet a further embodiment, the GRF peptide is selected from the group consisting of (a) a polypeptide comprising the amino acid
 10 sequence of SEQ ID NO: 3, (b) a polypeptide comprising the amino acid sequence of SEQ ID NO: 5; and (c) the polypeptide of (a) having a 1 to 14 amino acid deletion from its C-terminus. In another embodiment, the GRF analog is (hexenoyl trans-3)hGRF(1-44)NH₂ (SEQ ID NO: 7).

15 In another aspect, the invention provides a package comprising instructions for increasing muscle function. In an embodiment, the muscle function is selected from the group consisting of (a) muscle strength, (b) muscle endurance and (c) both (a) and (b). In an embodiment, the muscle function

is muscle strength, and in a further embodiment, peripheral muscle strength. In another embodiment, the muscle function is muscle endurance. In yet another embodiment, said increase in muscle function results in a reduction of a parameter selected from the group consisting of (a) breathing discomfort, (b) leg discomfort, and (c) both (a) and (b). In another embodiment, the increase in muscle function results in an increase in lean body mass in the subject and/or a decrease in fat mass in said subject. In a further embodiment, the subject suffers from wasting, and in a further embodiment, wasting is associated with a condition selected from the group consisting of chronic obstructive pulmonary disease (COPD), chronic renal failure, congestive heart failure, human immunodeficiency virus infection, acquired immunodeficiency syndrome, cancer, malnutrition, frailty, immobilization paraplegia and spinal disorder. In another embodiment, the subject suffers from severe wasting. In an embodiment, the subject has a body mass index less than or equal to 20, and/or a weight less than 90% of ideal body weight. In an embodiment, the subject is a male and has fat free mass index less than or equal to 16 or the subject is a female and has a fat free mass index less than or equal to 15. In another embodiment, the agent is adapted for an administration route selected from the group consisting of intravenous, oral, transdermal, subcutaneous, mucosal, intramuscular, intranasal, intrapulmonary, parenteral, intrarectal and topical. In yet another embodiment, the GH secretagogue is adapted for administration in a dose between about 0.0001 mg to about 4 mg, in a further embodiment, from about 0.0001 to about 2 mg, in a further embodiment, from about 1mg to about 2 mg, in a further embodiment, about 1 mg, in a further embodiment, about 2 mg.

20

In yet a further aspect, the present invention provides a composition for increasing muscle function in a subject, the composition comprising (a) a growth hormone (GH) secretagogue and (b) a pharmaceutically acceptable carrier.

5 In an embodiment, the GH secretagogue is selected from the group consisting of a GH-releasing factor (GRF) and a GRF analog. In a further embodiment, the GRF analog is a GRF analog of formula A:

10 X-GRF Peptide (A)

wherein;

the GRF peptide is a peptide of formula B;

15

A1-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-Tyr-Arg-Lys-A13-Leu-
A15-Gln-Leu-A18-Ala-Arg-Lys-Leu-Leu-A24-A25-Ile-A27-A28-
Arg-A30-R0 (B)

20 wherein,

A1 is Tyr or His;

A2 is Val or Ala;

A8 is Asn or Ser;

25 A13 is Val or Ile;

A15 is Ala or Gly;

A18 is Ser or Tyr;

A24 is Gln or His;

A25 is Asp or Glu;

30 A27 is Met, Ile or Nle

A28 is Ser or Asn;

A30 is a bond or amino acid sequence of 1 up to 15
residues; and

35 R0 is NH₂ or NH-(CH₂)_n-CONH₂, with n=1 to 12; and

X is a hydrophobic tail anchored via an amide bond to the N-terminus of the peptide and the hydrophobic tail defining a backbone of 5 to 7 atoms;

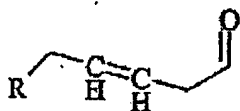
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wherein the backbone can be substituted by C₁₋₆ alkyl, C₃₋₆ cycloalkyl, or C₆₋₁₂ aryl and the backbone comprises at least one rigidifying moiety connected to at least two atoms of the backbone;

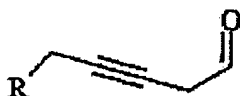
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said moiety selected from the group consisting of double bond, triple bond, saturated or unsaturated C₃₋₉ cycloalkyl, and C₆₋₁₂ aryl.

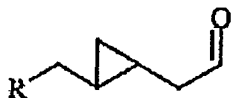
15 In another embodiment, X is selected from the group consisting of:



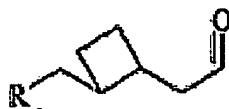
1 (R=H or CH₃ or CH₂CH₃)
cis or trans



2 (R=H or CH₃ or CH₂CH₃)



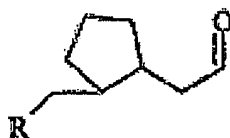
3 (R=H or CH₃ or CH₂CH₃)
cis or trans, both as racemic mixtures
or pure enantiomeric pairs



- 4** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, both as racemic mixtures
 or pure enantiomeric pairs



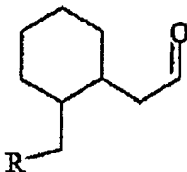
- 5** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, (when $R \neq H$)



- 6** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, both as racemic mixtures
 or pure enantiomeric pairs

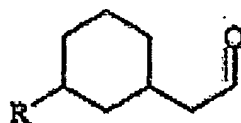
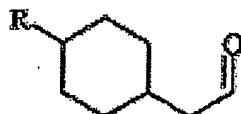
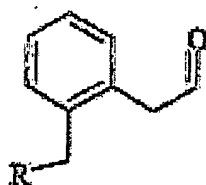
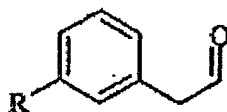
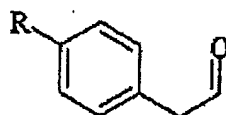
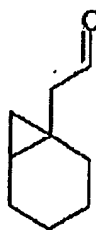


- 7** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, (when $R \neq H$)
 both as racemic mixtures
 or pure enantiomeric pairs



- 8** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, both as racemic mixtures
 or pure enantiomeric pairs

23

9 (R=H or CH₃ or CH₂CH₃)*cis* or *trans*, (when R ≠ H)both as racemic mixtures
or pure enantiomeric pairs10 (R=H or CH₃ or CH₂CH₃)*cis* or *trans*, (when R ≠ H)11 (R=H or CH₃ or CH₂CH₃)12 (R=H or CH₃ or CH₂CH₃)13 (R=H or CH₃ or CH₂CH₃) and

14

In a further embodiment, A30 is selected from the group consisting of (a) a bond, (b) an amino acid sequence corresponding to positions 30-44 of a natural GRF peptide, and (c) the amino acid sequence of (b) having a 1-14 amino acid deletion from its C-terminus. In yet another embodiment, the GRF peptide is selected from the group consisting of (a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 3, (b) a polypeptide comprising the amino acid sequence of SEQ ID NO: 5; and (c) the polypeptide of (a) having a 1 to 14 amino acid deletion from its C-terminus. In a further embodiment, the GRF analog is (hexenoyl trans-3)hGRF(1-44)NH₂ (SEQ ID NO: 7).

15 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Disposition of Subjects of the study presented herein.

Figure 2. Serum IGF-1 levels over the study period. Data are presented as mean \pm standard deviation (SD).

20 **Figure 3.** Changes in Lean Body Mass in the overall (A), low FFMI (B) and low BMI (C) populations. Data are presented as means \pm SD.

Figure 4. Changes in Fat Mass in the overall population. Data are presented as means \pm SD.

25 **Figure 5.** Changes over baseline in muscle strength (knee extension at 90°/sec.) in the overall (A), low FFMI (B) and low BMI (C) populations. Data are presented as means \pm SD.

Figure 6. Changes in Borg Scale for Breathing (A) and Leg (B) discomfort during the cycle ergometer test. Data are presented as means \pm SD.

5 DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a new use of a GH secretagogue, more specifically to a new use of GRF or its analogs. This invention also relates to a method for improving muscle function.

10 In a first aspect, this invention provides a method of increasing muscle function in a subject. In an embodiment, the method comprises administering an agent selected from the group consisting of a growth hormone (GH) secretagogue and a composition comprising a GH secretagogue
15 and a pharmaceutically acceptable carrier. "GH secretagogue" as used herein refers to any compound or molecule, natural or synthetic, which may result in, either directly or indirectly, GH secretion and/or an increase in GH secretion.

In embodiments, the GH secretagogue is a growth
20 hormone-releasing factor (GRF; also referred to as growth hormone releasing hormone [GHRH]) or a GRF analog.

In an embodiment, the GRF is human GRF (hGRF).

Human growth hormone-releasing factor (hGRF) is a peptide of 44 amino acids with a C-terminal NH₂ modification,
25 referred to herein as hGRF(1-44)NH₂, and has the following structure:

Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-
Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-

26

Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH₂
(SEQ ID NO: 2)

Therefore, the amino acid sequence of the just-
5 noted 44 amino acid form is as follows:

Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-
Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-
Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu (SEQ
10 ID NO: 3)

The minimum active core comprises the first 29
amino acids of the above sequence, which is referred to
herein as hGRF(1-29)NH₂, and has the following structure:

15 Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-
Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-NH₂
(SEQ ID NO: 4)

20 Therefore, the amino acid sequence of the just-
noted 29 amino acid form is as follows:

Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-
Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg (SEQ
25 ID NO: 5)

The 1-44 and 1-29 forms differ in that the 1-44
form contains the following additional amino acids, which
correspond to positions 30-44 of the 1-44 form:

30 Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu
(SEQ ID NO: 6)

27

In an embodiment, the GRF analog is a GRF analog of formula A:

X-GRF Peptide (A)

wherein;

the GRF peptide is a peptide of formula B;

A1-A2-Asp-Ala-Ile-Phe-Thr-A8-Ser-Tyr-Arg-Lys-A13-Leu-
A15-Gln-Leu-A18-Ala-Arg-Lys-Leu-Leu-A24-A25-Ile-A27-A28-
Arg-A30-R0 (B) (SEQ ID NO: 1)

wherein,

A1 is Tyr or His;

A2 is Val or Ala;

A8 is Asn or Ser;

A13 is Val or Ile;

A15 is Ala or Gly;

A18 is Ser or Tyr;

A24 is Gln or His;

A25 is Asp or Glu;

A27 is Met, Ile or Nle

A28 is Ser or Asn;

A30 is a bond or amino acid sequence of 1 up to 15
residues; and

R0 is NH₂ or NH-(CH₂)_n-CONH₂, with n=1 to 12; and

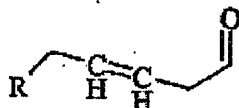
X is a hydrophobic tail anchored via an amide bond to
the N-terminus of the peptide and the hydrophobic tail
defining a backbone of 5 to 7 atoms;

wherein the backbone can be substituted by C₁₋₆ alkyl, C₃₋₆ cycloalkyl, or C₆₋₁₂ aryl and the backbone comprises at least one rigidifying moiety connected to at least two atoms of the backbone;

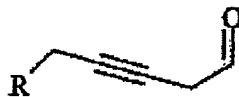
5

said moiety selected from the group consisting of double bond, triple bond, saturated or unsaturated C₃₋₉ cycloalkyl, and C₆₋₁₂ aryl.

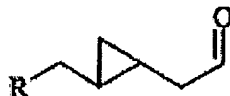
10 In embodiments, X noted above is selected from the group consisting of:



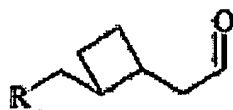
1 (R=H or CH₃ or CH₂CH₃)
cis or trans



2 (R=H or CH₃ or CH₂CH₃)



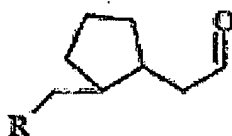
3 (R=H or CH₃ or CH₂CH₃)
cis or trans, both as racemic mixtures
or pure enantiomeric pairs



- 4** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, both as racemic mixtures
 or pure enantiomeric pairs



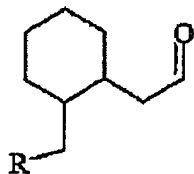
- 5** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, (when $R \neq H$)



- 6** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, both as racemic mixtures
 or pure enantiomeric pairs

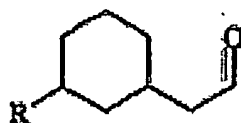
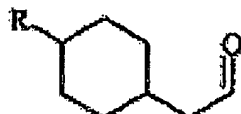
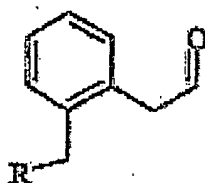
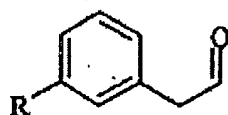
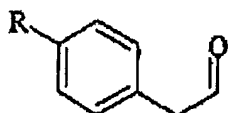


- 7** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, (when $R \neq H$)
 both as racemic mixtures
 or pure enantiomeric pairs



- 8** ($R=H$ or CH_3 or CH_2CH_3)
cis or *trans*, both as racemic mixtures
 or pure enantiomeric pairs

30

9 (R=H or CH₃ or CH₂CH₃)*cis* or *trans*, (when R ≠ H)both as racemic mixtures
or pure enantiomeric pairs10 (R=H or CH₃ or CH₂CH₃)*cis* or *trans*, (when R ≠ H)11 (R=H or CH₃ or CH₂CH₃)12 (R=H or CH₃ or CH₂CH₃)13 (R=H or CH₃ or CH₂CH₃)

14

In embodiments, A30 noted above is selected from the group consisting of a bond, an amino acid sequence corresponding to positions 30-44 of a natural GRF peptide and
5 said an amino acid sequence from the 30-44 fragment having a 1-14 amino acid deletion from its C-terminus.

In embodiments, the above-noted GRF peptide is selected from the group consisting of a polypeptide comprising the amino acid sequence of SEQ ID NO: 3; a
10 polypeptide comprising the amino acid sequence of SEQ ID NO: 5; and the polypeptide of (a) having a 1 to 14 amino acid deletion from its C-terminus.

In an embodiment, the above-noted GRF analog is (hexenoyl trans-3)hGRF(1-44)NH₂.

15 Methods of preparing the above-described GRF analogs are described in US patents No. 5,861,379 (Ibea et al., January 19, 1999); No. 6,020,311 (Brazeau et al., February 1, 2000), No. 6,458,764 (Gravel et al., October 1, 2002) and published US application No. 2004/0171534 A1
20 (Gravel et al., published September 2, 2004).

In embodiments, the invention also provides methods of treating a mammal, and further, a human.

In embodiments, the methods described herein also relate to improving muscle function. "Muscle function", as
25 used herein, is defined as the action for which a muscle is specially adapted, specialized or used. For example, pulmonary muscles, such as the diaphragm, are used to contract and expand the lungs to enable expiration and inspiration. As such, an increase in pulmonary muscle
30 function would result in increased breathing abilities, such

as an increase in pulmonary inspiratory pressure (PI_{max}). The determination of muscle function is specific to each muscle. Techniques for determining muscle function, such as the PI_{max} , are generally known to those skilled in the art (refer to
5 examples below).

The methods described herein relates to improving muscle strength. As used herein "muscle strength" is defined as the power to resist a force. Muscle strength can be measured by various techniques known to those skilled in the
10 art (refer to the examples below) ("Skeletal Muscle Dysfunction in Chronic Obstructive Pulmonary Disease", American Thoracic Society and European Respiratory Society Statement, AM J Respir Crit Care Med Vol 159, pp S1-S40, 1999). In an embodiment, muscle strength is peripheral muscle
15 strength (e.g. quadriceps strength).

In another embodiment, the methods described herein relate to improving muscle endurance. As used herein, "muscle endurance" is defined as the ability to sustain a prolonged stressful effort or activity. Techniques for
20 determining muscle endurance are specific to each muscle and are generally known to those skilled in the art (refer to the examples below). For an example of determination of muscle endurance, refer to "Measurement of Symptoms, Lung Hyperinflation, and Endurance during Exercise in Chronic
25 Obstructive Pulmonary Disease", Denis E. O'Donnell, Miu Lam, and Katherine A. Webb, Am J. Respir Crit Care Med 1998; 158:1557-1565.

In embodiments, the methods described herein result in a decrease in breathing discomfort and/or leg discomfort
30 (or leg fatigue).

In further embodiments, the methods result in an increase in lean body mass and/or a decrease in fat mass. Lean body mass refers the weight of the subject in the absence of fat. It thus includes the weight of bones, organs, muscles, etc. The fat mass is thus the remainder of the weight of the subject. The lean body weight can be estimated using the following formulas:

$$\text{For men} = (1.10 \times \text{Weight}(\text{kg})) - 128 \times (\text{Weight}^2 / (100 \times \text{Height}(\text{m})^2))$$

$$\text{For women} = (1.07 \times \text{Weight}(\text{kg})) - 148 \times (\text{Weight}^2 / (100 \times \text{Height}(\text{m})^2))$$

The lean body mass (and ultimately fat mass) can also be assessed quantitatively using dual X-ray absorptiometry (or DEXA) (Morabia et al., 1999).

In embodiments, the methods concern subjects who may be suffering from wasting. Wasting is a condition associated with a reduction in size of an organ or tissue. It is usually associated with lean mass loss, and more specifically muscle mass loss. It is also referred to as "cachexia" or "muscle depletion". Wasting may be associated with several conditions such as chronic obstructive pulmonary disease, chronic renal failure, congestive heart failure, HIV infection, AIDS, cancer, malnutrition, frailty (e.g. associated with aging), immobilization (e.g. due to a stroke), paraplegia, spinal disorders, etc. In a further embodiment, the subjects may be suffering from severe wasting (e.g. severe muscle depletion or severe cachexia). As used herein, "severe wasting" is associated with an excessive loss of muscle mass. This excessive loss of muscle mass can be observed typically in patients suffering from one or more listed above.

There are several well-recognized ways to define the body composition and, ultimately, the degree of muscle wasting or muscle depletion in subjects. In an embodiment, the degree of muscle depletion is measured by the involuntary weight loss over a defined period of time. In another embodiment, body composition is calculated as a percentage of ideal body weight (IBW). The IBW is defined as the ratio of the patient's weight over an ideal weight for his gender, age and height based on the "Metropolitan Life" tables. In a further embodiment, the body composition of a subject can be calculated by using Body Mass Index (BMI). The BMI is calculated using the following formula:

$$\text{BMI} = \frac{\text{Weight (in kg)}}{\text{Height}^2 \text{ (in meters)}}$$

Subjects having a BMI between 20 and 25 are considered to have a normal body weight. Subjects having a BMI lower than 20 are considered to have severe muscle depletion or to be suffering from wasting. Subjects having a BMI higher than 25 are considered overweight, and higher than 30, obese. In another embodiment, body composition is measure quantitatively by the dual energy x-ray absorptiometry (DEXA) technique ("Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition" Richard B. Mazess, Howard S. Barden, Joseph P. Bisek and James Hanson. Am J Clin Nutr 1990; 51:1106-12). This technique allows the determination of lean body mass, or fat-free-mass (sum of lean body mass and bone mineral mass) and the fat mass (total weight - fat-free mass). DEXA also allows the determination of the lean body mass index (LBMI) and the fat free mass index (FFMI) ("Physiologic Effects of Nutritional Support and Anabolic Steroids in Patients with Chronic Obstructive Pulmonary Disease" - Annemie M. W.J. Schols, Peter B. Soeters, Rob Mostert, Rob J. Pluymers, and

Emiel F.M. Wouters, AM J. Respir Crit Care Med 1995;
152:1268-74.).

It is generally reported in the art that patients are considered to have a severe wasting if their body mass index (BMI) is less than or equal to 20, or if their weight is less than or equal to 90% of ideal body weight, or if their fat free mass index (FFMI) is less than or equal to 16 (in men) or 15 (in women). These values can vary from one clinical condition to another.

As noted above, in various embodiments, the above-mentioned GH secretagogue may be used therapeutically in formulations or medicaments to effect the above-noted increase in muscle function and to prevent or treat the above-noted conditions. The invention provides corresponding methods of medical treatment, in which a therapeutic dose of a GH secretagogue is administered in a pharmacologically acceptable formulation, e.g. to a patient or subject in need thereof. Accordingly, the invention also provides therapeutic compositions comprising a GH secretagogue and a pharmacologically acceptable excipient or carrier. In one embodiment, such compositions include a GH secretagogue in a therapeutically or prophylactically effective amount sufficient to effect the above-noted increase in muscle function and to treat the above-noted conditions. The therapeutic composition may be soluble in an aqueous solution at a physiologically acceptable pH.

A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as to effect the above-noted increase in muscle function and to reduce the progression of the above-noted conditions. A therapeutically effective amount of a GH secretagogue may

vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also one in which any toxic or detrimental effects of the compound are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as preventing or inhibiting the rate of onset or progression of the above-noted conditions. A prophylactically effective amount can be determined as described above for the therapeutically effective amount. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions. In an embodiment, the GH secretagogue dosage is from about 0.0001 mg to about 4 mg, in a further embodiment, from about 0.0001 to about 2 mg, in a further embodiment, from about 1mg to about 2 mg, in a further embodiment, about 1 mg, in a further embodiment, about 2 mg.

As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Alternatively, the carrier can be suitable for intravenous, intraperitoneal, intramuscular, subcutaneous, sublingual or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile

injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage.

10 The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid

15 polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be

20 preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example,

25 monostearate salts and gelatin. Moreover, a GH secretagogue can be administered in a time release formulation, for example in a composition which includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as

30 a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen,

polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

5 Sterile injectable solutions can be prepared by incorporating the active compound (e.g. a GH secretagogue) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions
10 are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation
15 are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. In accordance with an alternative aspect of the invention, a GH secretagogue may be formulated with one or more additional
20 compounds that enhance its solubility.

In accordance with another aspect of the invention, therapeutic compositions of the present invention, comprising a GH secretagogue, may be provided in containers, kits or packages (e.g. commercial packages) which further comprise
25 instructions for its use to effect the above-noted increase in muscle function and to prevent or treat the above-noted conditions. The instructions may indicate that the uses result in a decrease in breathing discomfort and/or leg discomfort, a decrease in fat mass or an increase in lean
30 body mass.

Accordingly, the invention further provides a package (e.g. commercial package) comprising a GH

secretagogue or the above-mentioned composition together with instructions to effect the above-noted increase in muscle function and to prevent or treat the above-noted conditions.

The invention further provides use of a GH secretagogue to effect the above-noted increase in muscle function and to prevent or treat the above-noted conditions. The invention further provides use of a GH secretagogue for the preparation of a medicament to effect the above-noted increase in muscle function and to prevent or treat the above-noted conditions. The administration of the composition of the present invention can be from a route selected from the group consisting of oral, transdermal, intravenous, subcutaneous, mucosal, intramuscular, intranasal, intrapulmonary, parenteral, intrarectal and topical route. In embodiments, the above-noted instructions may indicate that the agent or composition may be administered subcutaneously. In further embodiments, the instructions may also indicate that the GH secretagogue dosage is administered in a dose from about 0.0001 mg to about 4 mg, in a further embodiment, from about 0.0001 to about 2 mg, in a further embodiment, from about 1mg to about 2 mg, in a further embodiment, about 1 mg, in a further embodiment, about 2 mg.

In another aspect, the invention also provides compositions comprising a GH secretagogue and a pharmaceutically acceptable carrier. In an embodiment, the composition can be used for improving muscle function, muscle strength and/or muscle endurance.

The results presented herein clearly show that administration of a GH secretagogue (such as the GRF analogue, TH9507), to patients suffering from wasting (e.g. COPD-associated wasting) significantly increased serum IGF-1

levels, augmented lean body mass, decreased fat mass and, surprisingly, improved muscle strength, muscle function and muscle endurance.

Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. In the claims, the word "comprising" is used as an open-ended term, substantially equivalent to the phrase "including, but not limited to". The following examples are illustrative of various aspects of the invention, and do not limit the broad aspects of the invention as disclosed herein.

EXAMPLES

Example 1 - Study drug

The compound used in the studies below is (hexenoyl trans-3)hGRF(1-44)NH₂ (also referred to as TH9507 herein), which is a synthetic human growth hormone releasing factor analog that comprises the 44-amino acid sequence of human growth hormone releasing factor (hGRF) on which a hexenoyl moiety, a C₆ side chain has been anchored on Tyr 1 at the NH₂-terminal. (hexenoyl trans-3)hGRF(1-44)NH₂ or TH9507 has the following structure:

(trans)CH₃-CH₂-CH=CH-CH₂-CO-Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-

41

Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH₂ (SEQ ID NO: 7)

(hexenoyl trans-3)hGRF(1-44)NH₂ was synthesized via the methods set forth in US 5,861,379 (Ibea et al.; January 19, 1999).

Example 2 - Material and methods

The results presented herein were obtained from a multicentre, randomized, double-blind, placebo-controlled study. The compound TH9507 was administered by subcutaneous injection daily (1 or 2 mg dose) for a three-month period. Patients were of both genders, aged 50 years or older, with stable COPD as per the 1995 American Thoracic Society criteria.

Body Mass Index (BMI) criteria. For subjects aged between 50 and 64, their BMI was less than or equal to 27 (kg/m²). For these subjects, if their BMI was between 23 and 27, their forced expiratory volume in one second (FEV1) is less than or equal to 50% of predicted value (Hankinson JL, Odencrantz JR, Fedan KP. Spirometric reference values from a sample of the general US population. AM J. Respir Crit Care Med. 1999; 159:179-187). For subjects aged 65 or older, their BMI was less than or equal to 28 (kg/m²). For these subjects, if their BMI was between 24 and 28, their forced expiratory volume in one second (FEV1) is less than or equal to 50% of predicted value (Hankinson JL, Odencrantz JR, Fedan KP. Spirometric reference values from a sample of the general US population. AM J. Respir Crit Care Med. 1999; 159:179-187).

Statistics. ANOVA was used to compare the changes from baseline at Month 1, 2 and 3. It also includes

comparisons between groups using Tukey adjustments. Repeated Measures Analyses were used to assess overall treatment effect (inter-group analyses) and overall time effect (within group analyses).

5 Population analyzed. Population were analyzed using ITT-LOCF (Intent To Treat-Last Observation Carried Forward). Subgroups have been created according to the following criteria: low Fat Free Mass Index (Low FFMI <15 for women, <16 for men), low BMI (BMI <20).

10 *Example 3 - Disposition of Subjects*

Adverse events and safety laboratory parameters (haematology, biochemistry, urinalysis) were measured using standard laboratory methods.

15 The disposition of subjects of the study presented herein are shown in Figure 1. The number of premature discontinuation was comparable in the 3 groups. None of the reasons for discontinuation was related to study drug except for one patient in the 2 mg group who showed elevated creatine phosphokinase (CPK) and serum glutamic oxaloacetic
20 transaminase (SGOT) values (*) following 1 month of treatment and that were considered to be possibly related to treatment.

Table 1 (below) presents the baseline characteristics of the subjects of the study.

Table 1. Baseline Characteristics. Data are presented as means \pm SD.

	Placebo	1 mg	2 mg
N	36	36	37
Women	8	7	7
Men	28	29	30
Age (years) (Min-Max)	65.5 \pm 8.6 (46.3 - 80.6)	64.7 \pm 7.6 (48.9 - 84.7)	63.4 \pm 8.4 (49.6 - 79.5)
BMI (kg/m²)	21.9 \pm 2.9	21.8 \pm 3.4	22.5 \pm 3.5
FEV1% pred. (Min-Max)	40.7 \pm 12.8 (22.2 - 66.8)	35.2 \pm 13.2 (15.9 - 63.6)	37.7 \pm 12.7 (12.4 - 63.2)
Median	37.9	35.0	37.6
IGF-1 (ng/mL)	115 \pm 39	115 \pm 44	112 \pm 34

Example 4 - Serum IGF-1 levels over the study period

5 Serum IGF-1 were measured after acid-ethanol extraction using the EsoterixTM RIA kit (Esoterix Inc., Calabasas Hill, CA) according to the manufacturer's instructions. The sensitivity of this kit is 10 ng/mL.

10 A significant increase over baseline was observed at each study time point in both TH9507-treated groups (*P < 0.001, Fig. 2) with the following changes at Month 3: placebo: -6%; 1 mg: +50%; 2 mg: +92%. Treatment effect was dose-related (P < 0.001) and significantly different when compared to placebo (P < 0.001) (Fig. 2.). Similarly,
15 significant increase in IGF-1 as compared to baseline and placebo were observed in the low FFMI and low BMI populations (+103% and +129% at Month 3, respectively).

Example 5 - Change in body composition

Body composition (lean body mass and fat mass) were assessed by Dual-Energy X-Ray Absorptiometry (DEXA).

Lean body mass. In the overall population, lean
5 body mass significantly increased at both doses when compared
to baseline and to placebo (*P < 0.001, Fig. 3A). Gains were
noticeable as early as Month 1. At Month 3, changes were as
follows: placebo: -0.1 kg; 1 mg: +1.3 kg; 2 mg: +0.9 kg. For
the low FFMI population, lean body mass significantly
10 increased at both doses when compared to baseline (*P <
0.001) and to placebo (P < 0.05) (Fig. 3B). At the end of the
treatment period, changes were as follows: placebo: -0.1 kg;
1 mg: +1.2 kg; 2 mg: +1.7 kg. For the low BMI population,
lean body mass increased at both doses when compared to
15 baseline (*P < 0.001) (Fig. 3C). At the end of the treatment
period, changes were as follows: placebo: -0.1 kg; 1 mg: +1
kg; 2 mg: +1.3 kg.

Fat mass. For the overall population, fat mass
significantly decreased at both doses when compared to
20 baseline (*P < 0.001, #P < 0.01) whereas an increase was
observed in the placebo group (+P < 0.05) (Fig. 4). Changes
were significant when compared to placebo but not between the
treated groups. At the end of the treatment period, changes
were as follows: placebo: +0.4 kg; 1 mg: -0.7 kg; 2 mg: -0.5
25 kg. Similarly, significant decreases in fat mass as compared
to baseline and placebo were observed in the low FFMI
(placebo: +0.1 kg; 1 mg: -1.1 kg; 2 mg: -0.9 kg - at Month 3)
and low BMI populations (placebo: +0.4 kg; 1 mg: -1.0 kg; 2
mg: -1.1 kg at Month 3).

Example 6 - Muscle function

Peripheral muscle strength was measured by isokinetic muscle strength testing. Briefly, in order to assess the quadriceps muscle strength, the subjects must
5 extend their knee at 90°/sec.

During an exercise performed with a cycle ergometer at 75% of maximal capacity, Borg scale at isotime for evaluation of dyspnea and leg discomfort were measured.

Muscle strength. For the overall population, muscle
10 strength significantly increased over baseline at 2 mg (*P < 0.05) (Fig. 5A). For the 1 mg dose, a trend was observed (P = 0.06) (Fig. 5A). For the low FFMI population, significant results over baseline were observed within both TH9507-
treated groups (*P < 0.05) (Fig. 5B). For the low BMI
15 population, muscle strength was significantly increased over baseline at 1 mg (+P < 0.01) while a trend was observed at 2 mg (P = 0.08) (Fig. 5C). Table 2 presents individual muscle strength results.

Table 2. Summary of individual changes in muscle strength (knee extension at 90°/sec). Data are percentage of subject with changes in muscle strength $\geq 10\%$ vs. baseline.

	Month 1 increase	Month 2 increase	Month 3 increase
Total Population			
Placebo	18.8%	28.1%	34.4%
1 mg	38.7%	38.7%	45.2%
2 mg	46.9%	37.5%	40.6%
Low BMI			
Placebo	0.0%	0.0%	25.0%
1 mg	57.1%	57.1%	57.1%
2 mg	44.4%	55.6%	55.6%
Low FFMI			
Placebo	0.0%	0.0%	30.0%
1 mg	40.0%	70.0%	70.0%
2 mg	44.4%	55.6%	44.4%

5 Both breathing and leg discomfort (or leg fatigue) Borg scales are significantly decreased over baseline at the 2 mg dose indicating an improvement in both symptoms (**P < 0.01) (Fig. 6A and B).

10 Throughout this application, various references are referred to describe more fully the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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